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(54) Title: POLYELECTROLYTE COMPLEX (E.G.ZWITTERIONIC POLYTHIOPHENES) WITH A RECEPTOR (E.G. POLYNUCLEOTIDE, ANTIBODY ETC.) FOR BIOSENSOR APPLICATIONS

(57) Abstract: The invention relates to a complex between a conjugated polyelectrolyte, and one or more receptor molecules specific for a target biomolecule analyte, said polyelectrolyte and said receptor being non-covalently bound to each other, usable as a probe for biomolecular interactions. It also relates to a method of determining selected properties of biomolecules. Thereby, a complex as above is exposed to a target biomolecule analyte whereby the analyte and the receptor interact, and a change of a property of said polyelectrolyte in response to the interaction between the receptor and the analyte is detected.The detected change is used to determine said selected property of said biomolecule.

Polyelectrolyte complex (e.g. zwitterionic polythiophenes) with a receptor (e.g. polynucleotide, antibody etc.) for biosensor applications

Field of the invention

The present invention relates to methods for detection of biomolecular interactions through the detection of alterations of the intra- and inter-chain processes in materials based on zwitterionic conjugated polyelectrolytes.

Background:

The development of materials that are capable of selectively detecting biomolecular interactions have come under increasing attention, owing to their large potential for molecular electronics and biosensors. In this regard, conjugated polymers (CPs) such as poly(thiophene) and poly(pyrrole) can be used to couple analyte/receptor interactions, as well as non-specific interactions, into observable responses. CPs based sensors are sensitive to very minor perturbations, due to amplification by a collective system response and therefore offer a key advantage compared to small molecules based sensors. The possibility to use CPs as detecting elements for biological molecules requires that polymers are compatible with aqueous environment. This has been accomplished by making conjugated (and sometimes luminescent) polyelectrolytes, as recently used to detect biomolecules through their impact on the conditions for photoinduced charge or excitation transfer. Conjugated polyelectrolytes offer possibilities for very sensitive measurements, and may become ubiquitous for genomics and proteomics in the future, if the optical or electronic processes in these materials can be used to track biospecific interactions.

The physical and chemical properties of conjugated polymers can be modified by the introduction of suitable side chains in the 3-position. Polythiophene derivatives that exhibit biotin and different carbohydrates has been synthesized and shown to undergo colorimetric transitions in response to binding of streptavidin and different types of bacteria and viruses, respectively. The presently demonstrated systems use covalent attachment of a receptor to the

Suitably the polyelectrolyte comprises copolymers or homopolymers of thiophene, pyrrole, aniline, furan, phenylene, vinylene or their substituted forms, and preferably the conjugated polyelectrolyte has one or more zwitterionic side chain functionalities.

In a further aspect of the invention, there is provided a biosensor device for determining selected properties of biomolecules, comprising a complex of the kind identified above, and a substrate for said complex in which said complex is exposable to said target analyte. The biosensor device is defined in claim 14.

In still another aspect of the invention there is provided a method of determining selected properties of biomolecules, comprising exposing a complex as defined above, to a target biomolecule analyte whereby the analyte and the receptor interact, detecting a change of a property of said polyelectrolyte in response to the interaction between the receptor and the analyte; and using the detected change to determine said selected property of said biomolecule. The method is defined in claim 17.

The multiplicity of biomolecular interactions that one may wish to identify also implies that the invention in a still further aspect, can be implemented in the form of a microarray, and which calls for anchoring and patterning of the detecting system on a surface, defined in claim 22..

Brief description of the drawings

Figure 1 shows the chemical structure of poly(3-[(S)-5-amino-5-carboxyl-3-oxapentyl]-2,5-thiophenylene hydrochloride) (POWT), a zwitterionic polythiophene derivative.

Figure 2 schematically illustrates the method according to the invention.

Figure 3 shows the absorptionspectra of 1.16 µmol POWT (on a monomer basis) and 0 mol (□), 6.4 nmol (◊) of an oligonucleotide (5´-CAT GAT TGA ACC ATC CAC CA-3´) after 5 minutes of incubation in 10 mM Na-phosphate buffer pH 7.5, or in the same buffer system with 6.4 nmol of a complementary oligonucleotide (Δ).

Figure 9 shows the microcontact printing of POWT. A square net of POWT on plasma etched polystyrene, with lines 25 µm wide surrounding the polystyrene squares of 100x100µm. Optical microscopy in reflected light.

Table 1 shows the difference in ratio of emission intensity at the wavelengths 540nm/585nm and 540nm/670nm upon addition of 1.28 nmol of different oligonucleotides to a mixture of 23.1 nmol POWT and 1.28 nmol of a single stranded oligonucleotide.

Table 2 shows the absorption maximum and the ratio of the intensity of the emitted light at 540nm/610nm for POWT and POWT/peptide complexes after 10 min incubation in 20 mM Na-phosphate pH7.4

Detailed description of the invention

In general terms, the present invention relates to a novel complex between zwitterionic conjugated polyelectrolytes and a receptor, the polyelectrolyte acting as a carrier for said receptor, without the requirement to label the analytes or to covalently attach the receptors to the carrier. The complex is used as a probe for responding to biomolecular interactions. It also relates to a biosensor device comprising such complex and a method for detection of molecular interactions.

The invention is based on zwitterionic polyelectrolyte forming a complex with one or more receptor molecules. This complex is formed without covalent bonding and is based on hydrogen bonding, electrostatic- and non-polar interactions between the zwitterionic conjugated polymers and the receptor molecules, herein referred to as non-covalent bonding, which further includes any type of bonding that is not covalent in its nature.

The present invention utilizes changes of the zwitterionic conjugated polyelectrolyte/receptor molecules complex or alterations of the net charge of the receptor molecules, which induce conformational transitions of the backbone of the zwitterionic conjugated polyelectrolyte, separation or aggregation of zwitterionic conjugated polyelectrolyte chains. Furthermore, conformational transitions of the backbone of the zwitterionic conjugated

processes within a polymer chain and the inter-chain processes are related to optical and electronic processes between adjacent polymer chains. This cause novel optical absorption and emission properties, due to the novel intra- and inter chain processes, that have not been seen for polycationic or polyanionic conjugated polyelectrolytes.

The functional groups of the zwitterionic side chain, charged anionic or cationic at different pH, make this polythiophene derivative suitable for forming polyelectrolyte complexes with negatively or positively charged oligomers and polymers. In addition, the zwitterionic groups create versatile hydrogen bonding patterns with different molecules.

The detailed description of the invention that follows will deal separately with the zwitterionic conjugated polyelectrolytes, receptor molecules, analytes, methods of detection, immobilization of conjugated polyelectrolytes and receptors, and arrays and lines. The invention is finally exemplified with a number of experiments demonstrating the utility thereof.

I Zwitterionic conjugated polymers

The present invention relates to a variety of conjugated polyelectrolytes, with a minimum of 5 mers, consisting of mers derived from the monomers thiophene, pyrrole, aniline, furan, phenylene, vinylene or their substituted forms, forming homopolymers and copolymers there from. Furthermore, monomers with anionic-, cationic or zwitterionic side chain functionalities are included within the scope of the invention. The side chain functionalities is derived from, but not limited to, amino acids, amino acid derivatives, neurotransmittors, monosaccharides, nucleic acids, or combinations and chemically modified derivatives thereof. The conjugated polyelectrolytes of the present invention may contain a single side chain functionality or may comprise two or more different side chain functionalities. The functional groups of the zwitterionic conjugated polyelectrolytes, charged anionic or cationic at different pHs, make these polyelectrolyte derivatives suitable for forming strong polyelectrolyte complexes with negatively or positively charged oligomers and polymers. In addition, the

altered intra- and intra chain processes of the zwitterionic conjugated polyelectrolyte.

Appropriate analytes include, but are not limited to, cells, viruses, bacteria, spores, microorganisms, peptides, carbohydrates, nucleic acids, lipids, pharmaceuticals, antigens, antibodies, proteins, enzymes, toxins, any organic polymers or combination of these molecules that interacts with receptors of interest.

The analytes can be chemically modified to interact with the receptor molecules of interest. Methods of derivatizing a diverse range of compounds (e. g. carbohydrates, proteins, nucleic acids and other chemical groups) are well known. For example, amino acid side chains can easily be modified to contain polar and non-polar groups, or groups with hydrogen bonding abilities.

IV Methods of detection

As already indicated, the present invention is based on the utilization of alterations of intra and inter chain processes of zwitterionic conjugated polyelectrolytes. These alterations can be observed by fluorescence, Förster resonance energy transfer (FRET), quenching of emitted light, absorption, impedance, refraction index, change in mass, visco-elastic properties, change in thickness or other physical properties. The conformational transitions of the backbone of the zwitterionic conjugated polyelectrolyte, separation or aggregation of polyelectrolyte chains will alter the intra- and inter-chain processes of the zwitterionic conjugated polyelectrolyte and can for example be detected as a change in the ratio of the intensities of the emitted light at two or more different wavelengths (see example 3). The emission intensities can be recorded by a fluorometer and enhancement of the photon flow in the detector can increase the sensitivity. This can be achieved using a laser as the excitation source.

The fluorometric change can also be detected by the use of a fluorescence microscope or a confocal microscope. A combination of excitation or emission

angstrom level. These techniques can thus be used for measuring alteration in intra- and inter chain processes of the zwitterionic conjugated polyelectrolytes.

The interaction of receptor and analyte molecules with zwitterionic conjugated polyelectrolyte can also be detected by electrical and electrochemical methods. A gel or network of the zwitterionic conjugated polyelectrolyte can be formed, and thus a three dimensional object is obtained where each polymer chain is in (indirect) contact with all chains in the network. If the zwitterionic conjugated polyelectrolyte is in a semiconducting state – such as when the luminescence properties is used – it will exhibit a rather low conductivity, which is somewhat difficult to easily distinguish from the ionic conductivity of the aqueous medium bathing the gel. It is therefore desirable to form highly conducting gels of the sensitive macromolecule that allow electrical conduction in the network. A difficulty is that the doping of the conjugated chains, which gives a metallic polymer and a high conductivity, will not only turn on conductivity but also change the mechanical properties and geometry of the chains, thereby hindering the mechanism at work in the case of luminescence detection. A solution to that problem is the use of two component polymer gels, where one component A gives the high conductivity and another component B the biospecific interactions. If these two compounds are combined in a suitable manner, the changes of geometry of the gel due to said interactions can be made to detect the interaction between component B and biomolecules. Component A can be an aqueous dispersion of a highly doped polymer and component B, the zwitterionic conjugated polyelectrolyte can be combined, to make gels. By measuring the DC or AC conductivity of these gels with two point and four point probe methods or by impedance spectroscopy, the change of conductance upon binding or exposure to analytes or net charge alteration of the receptors can be followed.

The intra- and inter-chain processes of the zwitterionic conjugated polyelectrolytes are altered by the interactions between receptor and analyte molecules or alteration of the net charge of the receptor molecule, and leads to changes of the electrochemical properties of the resulting complex, which can then be used to build electrochemical detectors for biomolecules. A change of the redox potential of the hydrogel formed in the presence of a biomolecule can

polyelectrolyte solution). Another way to immobilize the receptor molecules is to place them underneath or on top of the zwitterionic conjugated polyelectrolyte. Transfer of the receptor molecules mixed together with zwitterionic conjugated polyelectrolyte to the solid support can be achieved by, but not limited to, using dip coating, spin-coating, contact printing, screen printing, ink jet technologies, spraying, dispensing and microfluidic printing (see example 9) by the use of soft lithography (see example 10) or the Biacore™ system (see example 8). If the receptor molecules is to be placed underneath the zwitterionic zwitterionic conjugated polyelectrolyte it has to be transferred to the solid support in the same way as it would have been mixed together with the polyelectrolyte as mentioned above. Placing the receptor molecules on top of the zwitterionic conjugated polyelectrolyte is done in the same way but after the polyelectrolyte has been immobilized to the solid support. The receptor molecules will act as the recognition site for analytes or as anchors for performing enzymatic reactions, such as phosphorylation.

Solvents for the zwitterionic conjugated polyelectrolytes of the present invention and the receptor molecules during the immobilization to the solid support can be, but are not limited to, water, buffered water solutions, methanol, ethanol and combinations thereof. Supporting polymers of other kinds can also be added in this step.

When the receptor molecules are immobilized on the solid support underneath, on top of or together with the zwitterionic conjugated polyelectrolyte of the present invention they form a complex with the polyelectrolyte through non-covalent interactions (Figure 2). This complex is formed without covalent chemistry and is based on hydrogen bonding, electrostatic- and non-polar interactions between the zwitterionic conjugated polyelectrolyte and the receptor molecule. Immobilization of the receptors to the zwitterionic conjugated polyelectrolytes of the present invention may be desired to improve their ease of use, assembly into devices (*e.g.* arrays or parallel lines), stability, robustness, fluorescent response, to fit into the process of high-throughput-screening (HTS) using micro titer plates and other desired properties. While receptor molecules have been immobilized onto cationic or anionic conjugated polymers for detection of analytes [15], prior to the the present invention, immobilization

up the parallel analysis of one or different analytes to one or different receptors in an easy way. The main purpose of using arrays or lines is to increase ease of use, portability, quantification, selectivity among other qualities and characteristics. With this approach we can explore the ability to measure multicomponent samples and to use partially selective sensor spots. This gives the opportunity to analyse two or more samples of interest at the same time, to do on-chip concentration determinations and to study the background. By immobilizing the zwitterionic conjugated polyelectrolyte and/or the receptor molecules on solid supports of any size and in any chosen patterns (such as arrays, lines, spots, posts) small, portable, easily read and interpretable devices can be constructed.

The use of multiple arrays requires that detection can be done for a great number of biomolecules, more or less simultaneously. This is often done in the form of a microarray, where many individual detector elements (or probes) are integrated on a small surface area, to allow for massive parallelism in the detection. As we can construct each individual detector by the simple blending of the zwitterionic conjugated polyelectrolyte and biomolecules, we have removed the necessity of covalent chemistry for making each one of many thousands of detectors in a detector array (microarray). We have shown that the zwitterionic conjugated polyelectrolyte and zwitterionic conjugated polyelectrolyte/ biomolecule complexes can be printed by micro contact printing using elastomer stamps (Figure 9). Transfer onto a microarray surface may also be done by spotting zwitterionic conjugated polyelectrolyte solutions, or by ink jetting polyelectrolyte solutions or by the other methods mentioned above. These steps are essential to prepare a multipixel microarray.

Experimental

Example 1: Optical detection of DNA-hybridisation in solution.

A stock solution containing 0.5 mg ml^{-1} POWT in de-ionised water was prepared and incubated for 30 minutes. 50 µl of the polymer solution was mixed with 64 µl of DNA-solution (100 nmol ml^{-1}, 5´- CAT GAT TGA ACC ATC CAC CA -3´, purchased from SGSDNA, Köping, Sweden). After 15 minutes of incubation, the samples were diluted with de-ionised water, a stock buffer solution (Na-phosphate pH 7.5 and a 1.0 equivalent amount of the respective nucleotide (5´-

samples were diluted with de-ionised water, a stock buffer solution (Na-phosphate pH 7.5 and a 1.0 equivalent amount of the respective nucleotide (5´-TGG TGG ATG GTT CAA TCA TG-3´, 5´- TGG TGG ATG CTT CAA TCA TG -3´, 5´-TGG TGG AAC GTT CAA TCA TG -3´, 5´- TGG TGG AAC CTT CAA TCA TG -3´ or 5´- CAT GAT TGA ACC ATC CAC CA -3´, purchased from SGSDNA, Köping, Sweden) to a final volume of 1500 µl containing 10 mM Na-phosphate. The samples were incubated for 5 minutes and the emission spectra were recorded with a ISA Jobin-Yvon spex FluoroMax-2 apparatus. The difference in ratio of emission intensity at the wavelengths 540nm/585nm and 540nm/670nm were calculated. The emitted light at 540 nm and 585 nm is due to intra-chain processes and the emitted light at 670 nm is due to an inter-chain process (aggregation of POWT chains). Nucleotides with one, two or three mismatches can easily be detected, as the difference in ratio of the emission intensity at the wavelengths 540nm/585nm and 540nm/670nm are influenced by the degree of mismatch between the DNA strands (Table 1).

Table 1

Sequence	Difference in Ratio 540nm/585nm[a]	Difference in Ratio 540nm/670nm[a]
5´- CAT GAT TGA ACC ATC CAC CA – 3´ 3´- TGA CTA ACT TGG TAG GTG GT – 5´	0.000[b] ± 0.000	0.000[b] ± 0.000
5´- CAT GAT TGA ACC ATC CAC CA – 3´ 3´- TGA CTA ACT TCG TAG GTG GT – 5´	0.041[b] ± 0.003[c]	0.133[b] ± 0.013[c]
5´- CAT GAT TGA ACC ATC CAC CA – 3´ 3´- TGA CTA ACT TGC AAG GTG GT – 5´	0.052[b] ± 0.004[c]	0.219[b] ± 0.021[c]
5´- CAT GAT TGA ACC ATC CAC CA – 3´ 3´- TGA CTA ACT TCC AAG GTG GT – 5´	0.074[b] ± 0.007[c]	0.355[b] ± 0.034[c]

[a]The ratio of the intensity of the emitted light at 540 nm and 585 nm or 540 nm and 670 nm.

Table 2

	Absorption maximum (nm)	Ratio of the intensity of the emitted light at 540nm/610nm	Ratio of the intensity of the emitted light at 540nm/670nm
POWT	438	0.72	1.63
POWT + JR2E	451	0.24	0.44
POWT + JR2K	419	1.08	2.88
POWT + JR2E + JR2K	440	0.49	0.97

Example 5: Fluorescent detection of self-assembly of synthetic peptides in solution.

A stock solution containing 3.7 mg ml^{-1} POWT in de-ionised water was prepared and incubated for 30 minutes. 10 µl of the polymer solution was mixed with 10 µl or 20 µl of a negatively charged peptide (NH2-N-A-A-D-L-E-K-A-I-E-A-L-E-K-H-L-E-A-K-G-P-V-D-A-A-Q-L-E-K-Q-L-E-Q-A-F-E-A-F-E-R-A-G-COOH) or a positively charged peptide (NH2-N-A-A-D-L-K-K-A-I-K-A-L-K-K-H-L-K-A-K-G-P-V-D-A-A-Q-L-K-K-Q-L-K-Q-A-F-K-A-F-K-R-A-G-COOH) solution (2.2 mg ml^{-1}), respectively and diluted with de-ionised water to a final volume of 300 µl. After 15 minutes of incubation, the samples were diluted with a stock buffer solution (Na-phosphate pH 7.4) and 10 µl de-ionised water or 10 µl of the positive/negative peptide solution (2.2 mg ml^{-1}) to a final volume of 2000 µl containing 20 mM Na-phosphate. The samples were incubated for 10 minutes in room temperature and the emission spectra (Figure 5, Table 2) were recorded with an ISA Jobin-Yvon spex FluoroMax-2 apparatus. Addition of JR2K will shift the emission maximum to shorter wavelengths and increase the intensity of the emitted light, indicative of a non-planar POWT backbone and separation of POWT chains, and addition of JR2E will shift the emission maximum to longer wavelengths and decrease the intensity of emitted light, indicative of a planar POWT backbone and aggregation of POWT chain (figure 5). JR2E and JR2K has been tailor made to form a four-helix bundle and the formation of this structure

solution (10 mM Na-phosphate pH 7.5) with 10 nmol 5´- AGA TTG GCG CAT TAC GAG GTT AGA -3´ (purchased from SGSDNA, Köping, Sweden) for 2 h. The fluorescence from the spots was recorded with an epifluorescence microscope (Zeiss Axiovert inverted microscope A200 Mot) equipped with a CCD camera (Axiocam HR), using a 405/30 nm bandpass filter (LP450, exposure time: 1500 ms), a 470/40nm bandpass filter (LP515, exposure time: 1500 ms) and a 546/12 nm bandpass filter (LP590, exposure time: 500 ms) .The alterations of the intra- and interchain processes of POWT, due to DNA-hybridisation, are seen as a change of the colour and the intensity of the emitted light from POWT (figure 7).

Example 8: Detection of DNA-hybridisation on a surface by surface plasmon resonance (SRP).

A bare gold sensor chip was spin casted (1000 rpm, 30 s) with a 5 mg/ml solution of POWT in milliQ water. The film were annealed by heating the chip at 75 °C for 5 min. Finally, the chip was assembled on the sensor chip support by using glue or adhesive strips. Generally an injection sequence consisting of three injections were performed. The first injection aims to characterize the polymer with ssDNA(5´-AGA TTG GCG CAT TAC GAG GTT AGA-3´, purchased from SGSDNA, Köping, Sweden), the second to verify that no unspecific binding occurs and the final injection aims to prove specific binding in the form of DNA hybridisation using 5´-AGA TTG GCG CAT TAC GAG GTT AGA-3´ or 5´-TCT AAC CTC GTA ATG CGC CAA TCT-3´ (purchased from SGSDNA, Köping, Sweden), respectively. The polymer films were first swollen in degassed milliQ water and then equilibrated in degassed 20 mM phosphate pH 7,4 buffer (PBS) with salt concentrations (NaCl) ranging from 0 to 1 M. The injected DNA was solved in the same buffer as the running buffer and the concentration was usually around 1 µM. The temperature was set to 25 °C during all experiments. The hybridisation event was monitored with a BiacoreX instrument from Biacore AB (Uppsala, Sweden). The instrument has two flow channels with the approximate size of 0.5×2.5 mm. Manual loading is required and the maximal injection volume is 100 µl. As shown in figure 8, a huge increase of the response unit (RU) is detected after injection of a DNA strand complementary to the target strand (receptor). The response unit is just slightly altered by the injection of non-complementary DNA, clearly showing that DNA-hybridisation is detected.

commercial name Baytron from Bayer AG) is deposited. The zwitterionic polymer/oligonucleotide complex is transferred on to the polymer surface by solution casting, contact printing, ink-jet printing or in other ways. The resulting layer is analyzed using 2- or 4-point resistance measurement, by electrochemical methods or by impedance spectroscopy.

CLAIMS:

1. A complex between a conjugated polyelectrolyte, and one or more receptor molecules specific for a target biomolecule analyte, said polyelectrolyte and said receptor being non-covalently bound to each other, usable as a probe for biomolecular interactions.

2. The complex as claimed in claim 1, wherein the polyelectrolyte comprises copolymers or homopolymers of thiophene, pyrrole, aniline, furan, phenylene, vinylene or their substituted forms.

3. The complex as claimed in claim 1 or 2, wherein said conjugated polyelectrolyte has one or more zwitterionic side chain functionalities.

4. The complex as claimed in claim 3, wherein said zwitterionic side chain functionalities comprise amino acids, amino acid derivatives, neurotransmittors, monosaccharides, nucleic acids, or combinations and chemically modified derivatives thereof.

5. The complex as claimed in claim 3 or 4, wherein the zwitterionic functionalities comprise one or more anionic and cationic side chain functionalities.

6. The complex as claimed in claim 1, wherein said receptor molecules are selected from the group consisting of peptides, carbohydrates, nucleic acids, lipids, pharmaceuticals, antigens, antibodies, proteins, organic polymers or combination of these molecules capable of interacting with said target analyte.

7. The complex as claimed in claim 1, wherein said conjugated polyelectrolyte is confined, adsorbed or covalently attached to a solid support.

8. The complex as claimed in claim 1, wherein said conjugated polyelectrolyte is in solution.

15. A biosensor device as claimed in claim 14, wherein said biosensor device comprises a receptacle and the polyelectrolyte is immobilized on a surface of said receptacle.

16. A biosensor device as claimed in claim 14, wherein said receptacle is a flow cell.

17. A method of determining selected properties of biomolecules, comprising:

exposing a complex as claimed in any of claims 1- 13, to a target biomolecule analyte whereby the analyte and the receptor interact,

detecting a change of a property of said polyelectrolyte in response to the interaction between the receptor and the analyte; and

using the detected change to determine said selected property of said biomolecule.

18. The method as claimed in claim 17, wherein the change of said property is detected by measuring fluorescence, Förster resonance energy transfer (FRET), quenching of emitted light, absorption, impedance, refraction index, mass, visco-elastic properties, thickness or other physical properties.

19. A method of manufacturing a biosensor device, wherein a complex as claimed in any of claims 1-13 is attached to a substrate surface, preferably in a suitable receptacle.

20. The method as claimed in claim 19, wherein the complex is attached by

i) transferring a conjugated polyelectrolyte to said surface by a method selected from dip coating, spin-coating, contact printing, screen printing, ink jet technologies, spraying, dispensing and microfluidic printing by the use of soft lithography, or combinations thereof, and

ii) forming a complex between a suitable receptor and said polyeletrolyte.

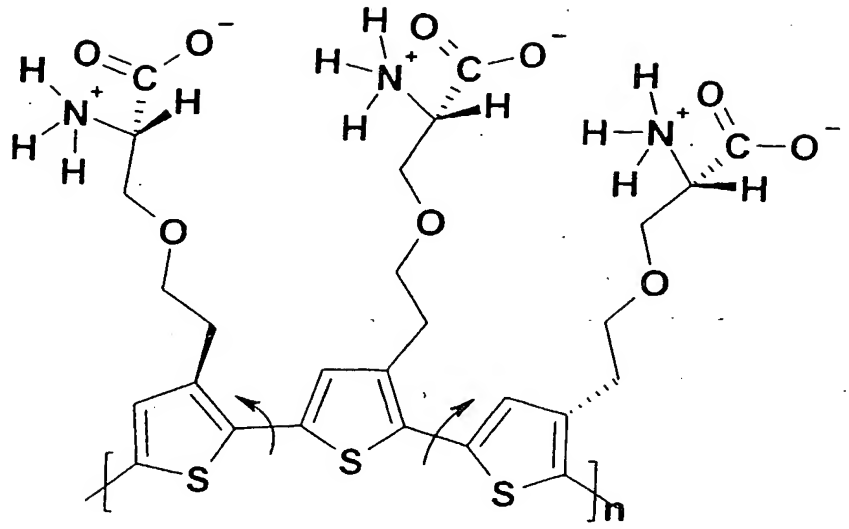


Fig. 1

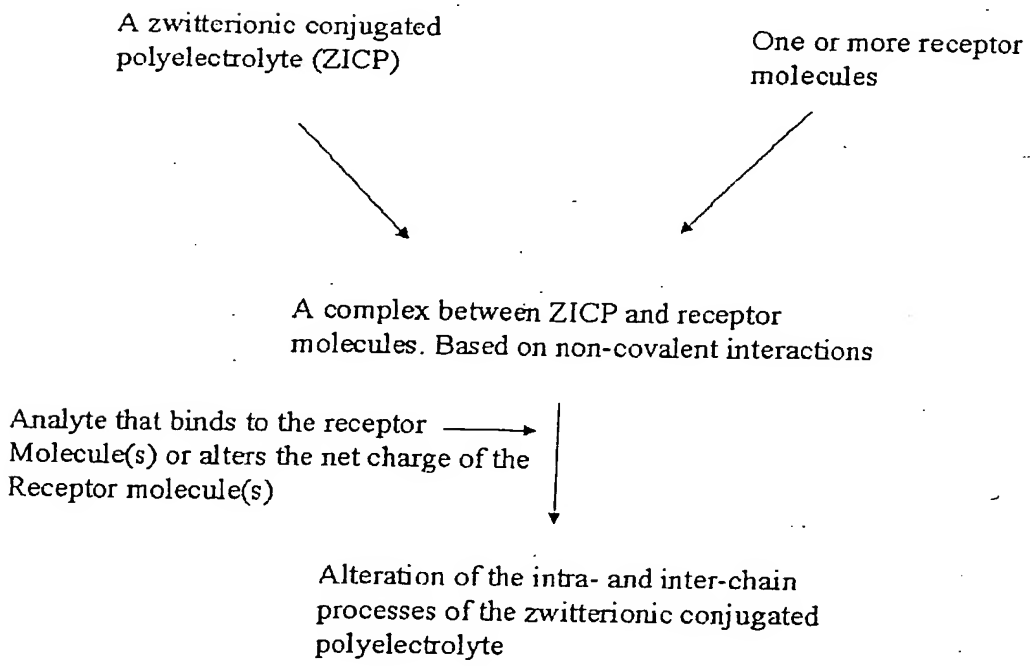
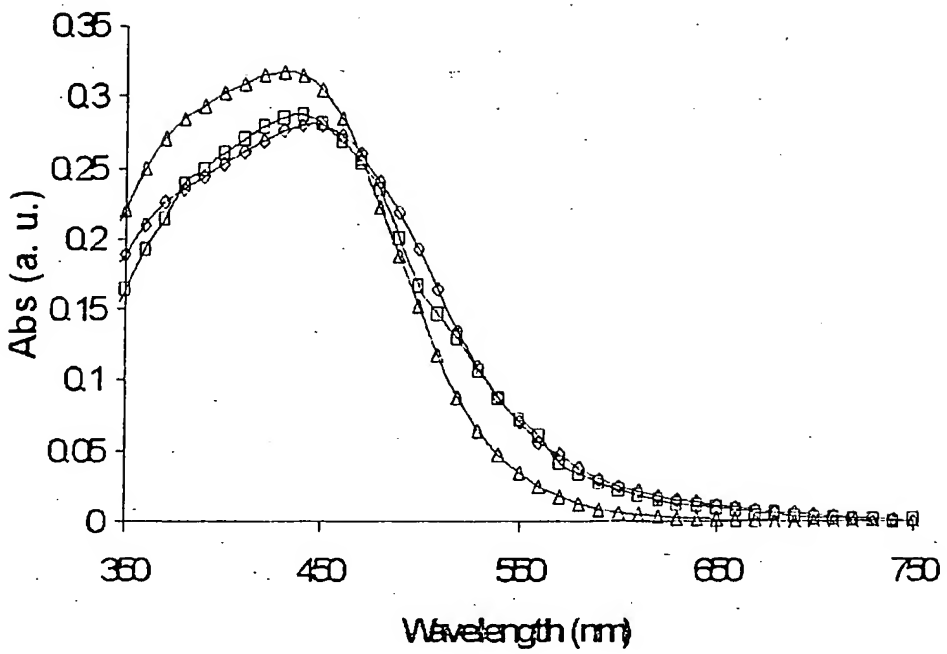

A zwitterionic conjugated polyelectrolyte (ZICP)
One or more receptor molecules
A complex between ZICP and receptor molecules. Based on non-covalent interactions
Analyte that binds to the receptor Molecule(s) or alters the net charge of the Receptor molecule(s)
Alteration of the intra- and inter-chain processes of the zwitterionic conjugated polyelectrolyte

Fig. 2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Abs (a. u.)
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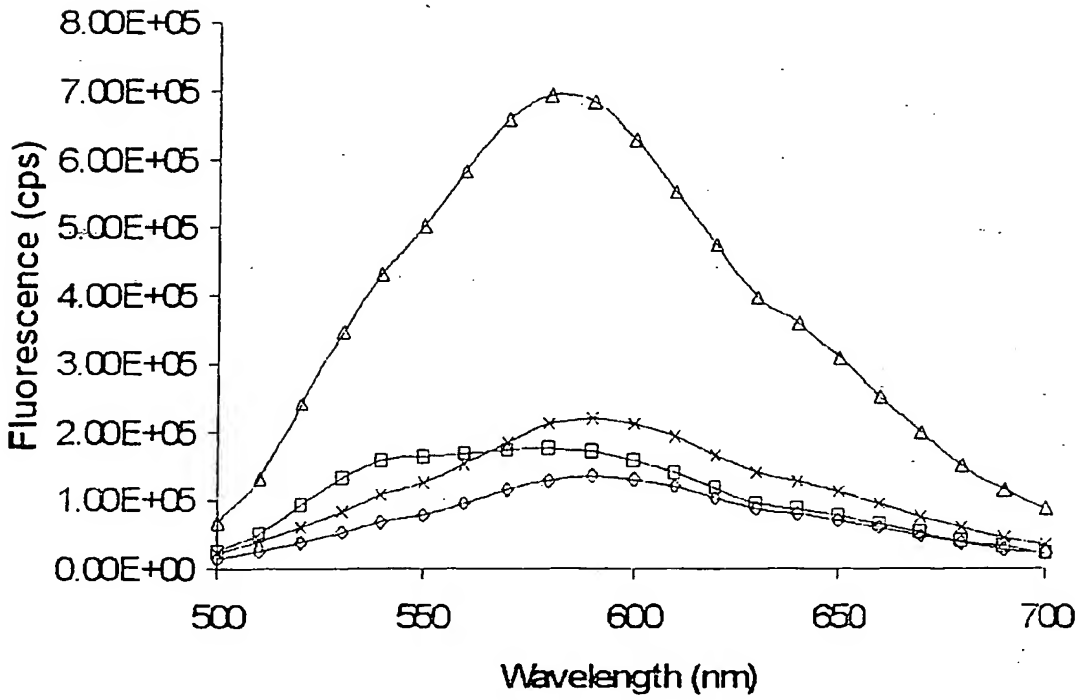
550
650
750
Wavelength (nm)

Fig. 3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Fluorescence (cps)
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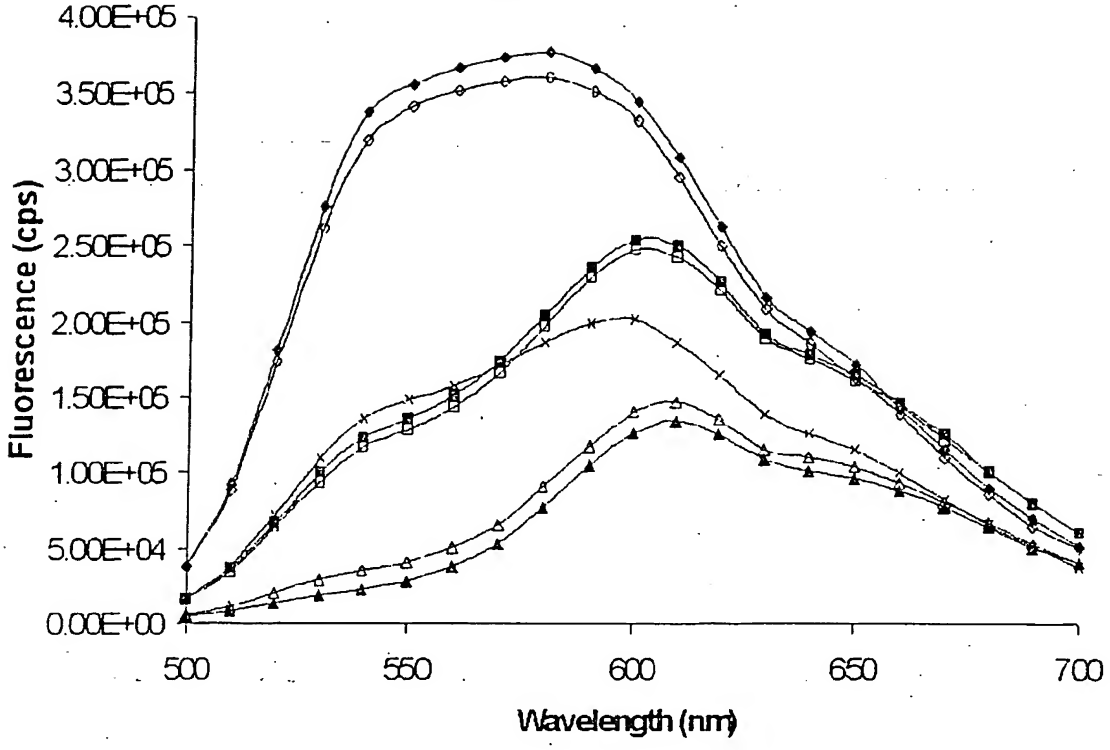
700
Wavelength (nm)

Fig. 4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3.00E+05
2.50E+05
2.00E+05
1.50E+05
1.00E+05
5.00E+04
0.00E+00
Fluorescence (cps)
500
550
600
650
700
Wavelength (nm)

Fig. 5

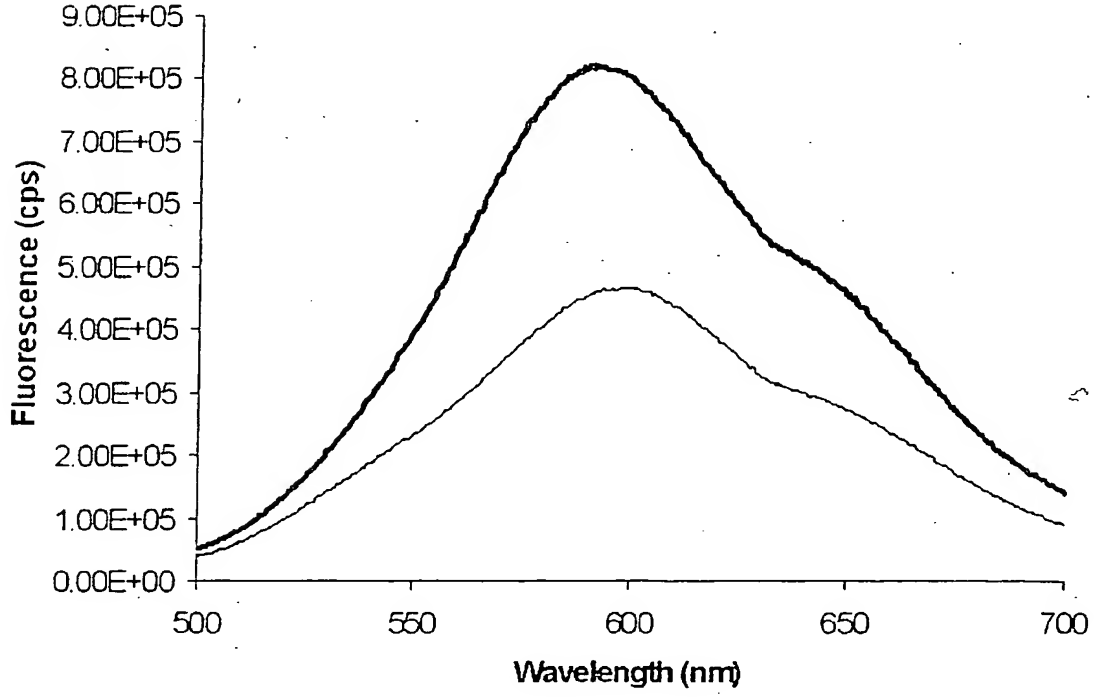
9.00E+05
8.00E+05
7.00E+05
6.00E+05
5.00E+05
4.00E+05
3.00E+05
2.00E+05
1.00E+05
0.00E+00
Fluorescence (cps)
500
550
600
650
700
Wavelength (nm)

Fig. 6

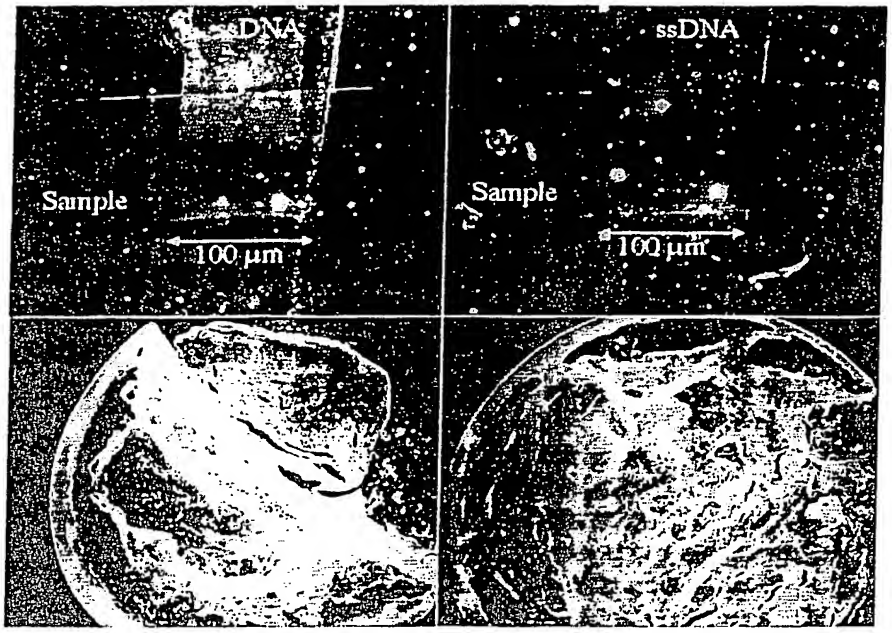

Fig. 7

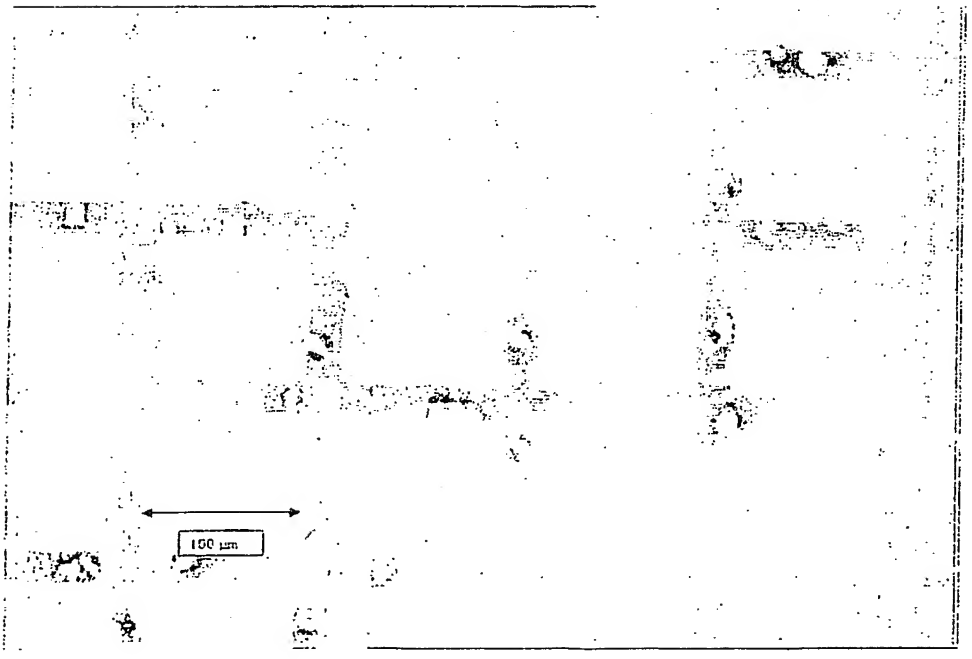

Fig. 9

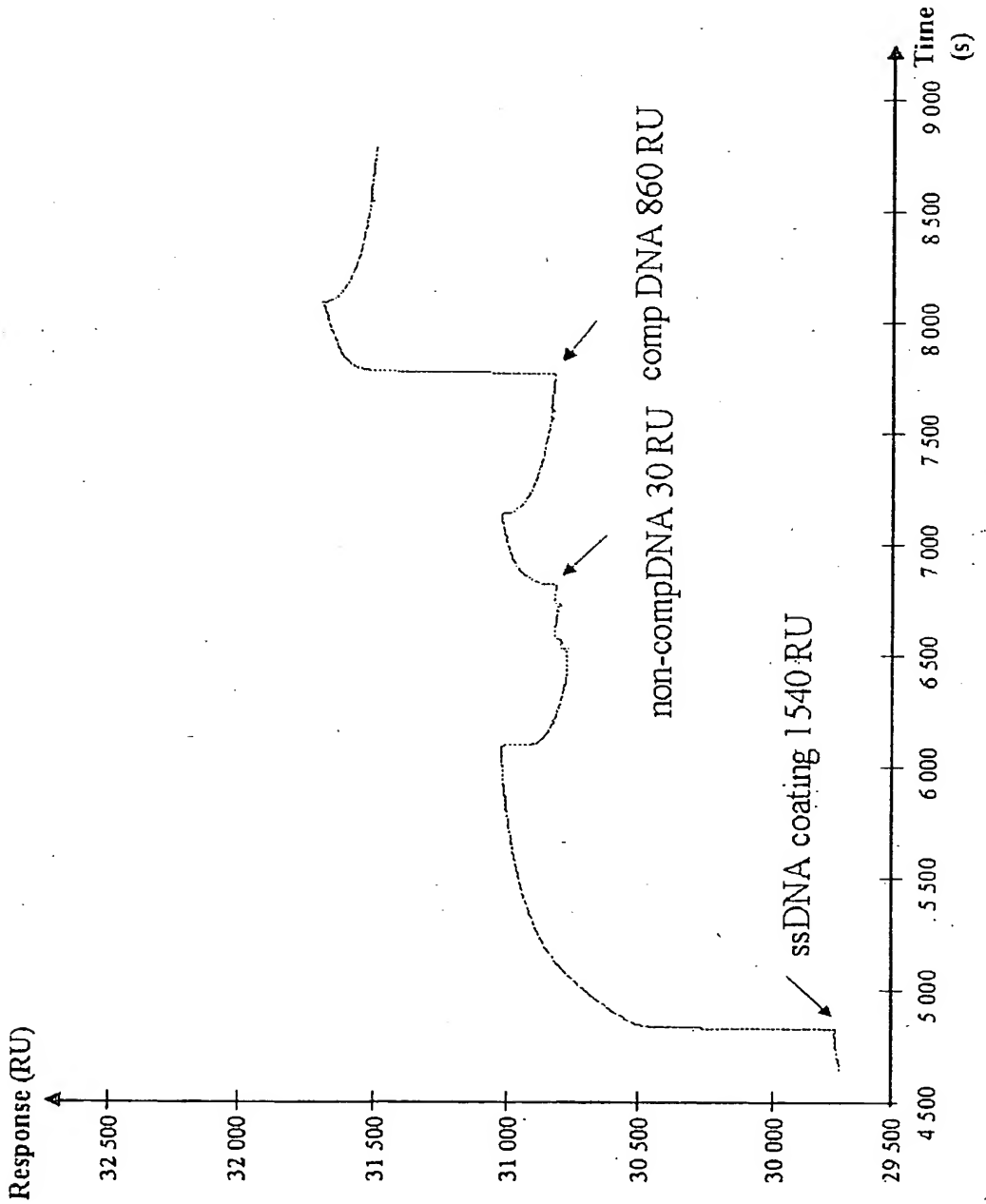
Response (RU)
32 500
32 000
31 500
31 000
30 500
30 000
29 500
4 500
5 000
5 500
6 000
6 500
7 000
7 500
8 000
8 500
9 000
Time (s)
ssDNA coating 1540 RU
non-compDNA 30 RU
comp DNA 860 RU

Fig. 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 03/00762

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: G01N 33/53, C12Q 1/68, G01N 33/543
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12Q, G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL, CHEMICAL ABSTRACTS, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9967423 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA), 29 December 1999 (29.12.99), see pages 36-41, examples 4,5 and 7, claims 12,21, 37 and 46, figures 37-41 --	1-23
X	US 2001026915 A1 (DEBORAH J. CHARYCH ET AL), 4 October 2001 (04.10.01), page 12 (0169) - page 16 (0184), figure 4 --	1-23
X	US 6096825 A (FRANCIS GARNIER), 1 August 2000 (01.08.00), example 3, column 6, figures 2,3 --	1-23

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 August 2003	28 -08- 2003
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. + 46 8 666 02 86	Authorized officer Carl-Olof Gustafsson/EÖ Telephone No. + 46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

Int[illegible]nal application No.
PCT/SE03/00762

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-23
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see next sheet

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 03/00762

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9967423 A1	29/12/99	AU 748644 B	06/06/02
		AU 4704799 A	10/01/00
		CA 2330937 A	29/12/99
		EP 1112377 A	04/07/01
		US 6306598 B	23/10/01
		US 2001026915 A	04/10/01
US 2001026915 A1	04/10/01	US 6395561 B	28/05/02
		AU 715973 B	10/02/00
		AU 1842297 A	20/08/97
		BR 9707207 A	20/07/99
		CA 2244098 A	31/07/97
		EP 0883690 A	16/12/98
		IL 125517 D	00/00/00
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		WO 9727316 A	31/07/97
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		AU 714839 B	13/01/00
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		EA 409 B	00/00/00
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		WO 9839632 A	11/09/98
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		EP 1112377 A	04/07/01
		WO 9967423 A	29/12/99

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International Bureau

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(81) Designated States *(national)*: AE, AG, AL, AM, AT (utility model), AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ (utility model), CZ, DE (utility model), DE, DK (utility model), DK, DM, DZ, EC, EE (utility model), EE, ES, FI (utility model), FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK (utility model), SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/096016 A1

(54) Title: POLYELECTROLYTE COMPLEX (E.G.ZWITTERIONIC POLYTHIOPHENES) WITH A RECEPTOR (E.G. POLYNUCLEOTIDE, ANTIBODY ETC.) FOR BIOSENSOR APPLICATIONS

(57) Abstract: The invention relates to a complex between a conjugated polyelectrolyte, and one or more receptor molecules specific for a target biomolecule analyte, said polyelectrolyte and said receptor being non-covalently bound to each other, usable as a probe for biomolecular interactions. It also relates to a method of determining selected properties of biomolecules. Thereby, a complex as above is exposed to a target biomolecule analyte whereby the analyte and the receptor interact, and a change of a property of said polyelectrolyte in response to the interaction between the receptor and the analyte is detected.The detected change is used to determine said selected property of said biomolecule.